



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C12N 9/10, 15/82, A01H 5/06	A1	(11) International Publication Number: WO 97/20040 (43) International Publication Date: 5 June 1997 (05.06.97)
(21) International Application Number: PCT/SE96/01558 (22) International Filing Date: 28 November 1996 (28.11.96) (30) Priority Data: 9504272-7 29 November 1995 (29.11.95) SE 9601506-0 19 April 1996 (19.04.96) SE (71) Applicant (for all designated States except US): AMYLOGENE HB [SE/SE]; c/o Svalöf Weibull AB, S-268 81 Svalöv (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): EK, Bo [SE/SE]; Nyhagen, S-740 30 Björklinge (SE). KHOSNOODI, Jamshid [SE/SE]; Bandstolsvägen 3, 2 tr., S-756 48 Uppsala (SE). LARSSON, Clas-Tomas [SE/SE]; Flogstavägen 55 B II, S-752 73 Uppsala (SE). LARSSON, Håkan [SE/SE]; Hammarbygatan 58, S-753 24 Uppsala (SE). RASK, Lars [SE/SE]; Säves väg 14, S-752 63 Uppsala (SE). (74) Agent: AWAPATENT AB; P.O. Box 5117, S-200 71 Malmö (SE).		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: STARCH BRANCHING ENZYME II OF POTATO		
(57) Abstract		
<p>The present invention relates to an amino acid sequence of second starch branching enzyme (SBE II) of potato and a fragment thereof as well as to the corresponding isolated DNA sequences. Furthermore, the invention relates to vectors comprising such an isolated DNA sequence, to processes for production of transgenic potatoes, and to the use of said potatoes for the production of starch. The starch obtained will show a changed pattern of branching of amylopectin as well as a changed amylose/amylopectin ratio.</p>		

STARCH BRANCHING ENZYME II OF POTATO

The present invention relates to a novel starch branching enzyme of potato. More specifically, the present invention relates to an amino acid sequence of a second starch branching enzyme (SBE II) of potato and a fragment thereof as well as their corresponding DNA sequences. Furthermore, the invention relates to vectors comprising such DNA sequences, to processes for production of transgenic potatoes, and to the use of said potatoes for the production of starch.

Starch is a complex mixture of different molecule forms differing in degree of polymerization and branching of the glucose chains. Starch consists of amylose and amylopectin, whereby the amylose consists of an essentially linear α -1,4-glucan and amylopectin consists of α -1,4-glucans connected to each other via α -1,6-linkages and, thus, forming a branched polyglucan. Thus, starch is not a uniform raw material.

Starch is synthesized via at least three enzymatic reactions in which ADP glucose phosphorylase (EC 2.7.7.27), starch synthase (EC 2.4.1.21) and starch branching enzyme (EC 2.4.1.18) are involved. Starch branching enzyme (SBE, also called Q-enzyme) is believed to have two different enzymatic activities. It catalyzes both the hydrolysis of α -1,4-glucosidic bonds and the formation of α -1,6-glucosidic bonds during synthesis of the branched component in starch, i.e. amylopectin.

Plant starch is a valuable source of renewable raw material used in, for example, the chemical industry (Visser and Jacobsen, 1993). However, the quality of the starch has to meet the demands of the processing industry wherein uniformity of structure is an important criterion. For industrial application there is a need of plants only containing amylose starch and plants only containing amylopectin starch, respectively.

fragments thereof, which after insertion into the genome of the plants cause changes in said branching degree and ratio in regenerated plants.

Within the scope of the present invention there is also included the amino acid sequence of SBE II and fragments thereof.

Also variants of the above DNA sequence resulting from the degeneracy of the genetic code are encompassed.

The novel DNA sequence encoding SBEII, comprising 3074 nucleotides, as well as the corresponding amino acid sequence comprising 878 amino acids, are shown in SEQ ID No. 1. One 1393 nucleotides long fragment of the above DNA sequence, corresponding to nucleotides 1007 to 2399 of the DNA sequence in SEQ ID No. 1, as well as the corresponding amino acid sequence comprising 464 amino acids, are shown in SEQ ID No. 2.

Furthermore, there are provided vectors comprising said isolated DNA-sequences and regulatory elements active in potato. The DNA sequences may be inserted in the sense or antisense (reversed) orientation in the vectors in relation to a promoter immediately upstream from the DNA sequence.

Also there is provided a process for the production of transgenic potatoes with a reduced degree of branching of amylopectin starch, comprising the following steps:

- a) transfer and incorporation of a vector according to the invention into the genome of a potato cell, and
- b) regeneration of intact, whole plants from the transformed cells.

Finally, the invention provides the use of said transgenic potatoes for the production of starch.

The invention will be described in more detail below in association with an experimental part and the accompanying drawings, in which

Fig. 1 shows SDS polyacrylamide electrophoresis of proteins extracted from starch of normal potato (lane A)

dissolved in 50 mM Tris-HCl, pH 7.5. An aliquot of each preparation was analyzed by SDS poly-acrylamide gel electrophoresis according to Laemmli (1970) (Fig. 1). The proteins in the remaining portions of the preparations were concentrated by precipitation with trichloroacetic acid (10%) and the proteins were separated on an 8% SDS polyacrylamide gel Laemmli, (1970). The proteins in the gel were stained with Coomassie Brilliant Blue R-250 (0.2% in 20% methanol, 0.5% acetic acid, 79.5% H₂O).

10 *In gel digestion and sequencing of peptides*

The stained bands marked with arrows in Fig. 1 corresponding to an apparent molecular weight of about 100 kDa were excised and washed twice with 0.2M NH₄HCO₃ in 50% acetonitrile under continuous stirring at 35°C for 20 min. After each washing, the liquid was removed and the gel pieces were allowed to dry by evaporation in a fume hood. The completely dried gel pieces were then separately placed on parafilm and 2 µl of 0.2M NH₄CO₃, 0.02% Tween-20 were added. Modified trypsin (Promega, Madison, WI, USA) (0.25 µg in 2 µl) was sucked into the gel pieces whereafter 0.2M NH₄CO₃ was added in 5 µl portions until they had resumed their original sizes. The gel slices were further divided into three pieces and transferred to an Eppendorf tube. 0.2M NH₄CO₃ (200 µl) was added and the proteins contained in the gel pieces were digested over night at 37°C (Rosenfeld et al. 1992). After completed digestion, trifluoroacetic acid was added to 1% and the supernatants removed and saved. The gel pieces were further extracted twice with 60% acetonitrile, 0.1% trifluoroacetic acid (200 µl) under continuous shaking at 37°C for 20 min. The two supernatants from these extractions were combined with the first supernatant. The gel pieces were finally washed with 60% acetonitrile, 0.1% trifluoroacetic acid, 0.02% Tween-20 (200 µl). Also these supernatants were combined with the other supernatants and the volume was reduced to 50 µl by evaporation. The

Purification of mRNA from potato tuber, synthesis of cDNA and PCR amplification of a cDNA fragment corresponding to potato starch branching enzyme II.

Total RNA from mature potato tubers (*S. tuberosum* cv. Amanda) was isolated as described (Logemann et al. 1987). First strand cDNA was synthesized using 2 µg of total RNA and 60 pmol of oligo-dT₃₀ as downstream primer. The primer was annealed to the polyA of the mRNA at 60°C for 5 min. The extension of the cDNA was performed according to the technical manual of the manufacturer using the Riboclone[®] cDNA Synthesis System M-MLV (H-) (Promega).

cDNA encoding the novel starch branching enzyme II according to the invention was amplified in a Perkin-Elmer GeneAmp[®] 9600 PCR thermocycler (Perkin-Elmer Cetus Instruments, CT, USA) using the two degenerate primers designed from the peptides 1 and 2 (see above) under the following conditions: 1 mM dNTP, 1 µM of each primer and an alicot of the cDNA described above in a total reaction volume of 20 µl with 1x AmpliTaq[®] buffer and 0,8 U AmpliTaq[®] (Perkin-Elmer Cetus). The cycling conditions were: 96°C for 1', 80°C while the enzyme was added as a hotstart (approximately 15'), an unintended drop to 25°C, five cycles of 94°C for 20", 45°C for 1', ramp to 72°C for 1' and 72°C for 2', and 30 cycles of 94°C for 5", 45°C for 30", and 72°C for (2'+2" per cycle) and completed with 72°C for 10' prior to chilling to 4°C.

A sample of this reaction (0.1 µl) was reamplified using the cycling conditions: 96°C for 1', 80°C while the enzyme was added as a hotstart (approximately 5'), five cycles of 94°C for 20'', 45°C for 1', and 72°C for 2', and 25 cycles of 94°C for 5'', 45°C for 30'', and 72°C for (2' + 2'' per cycle) and completed with 72°C for 10' prior to chilling to 4°C. After completion of the PCR amplification, the reaction was loaded on a 1.5% Seakem[®] agarose gel (FMC Bioproducts, Rockland, ME, USA). After electrophoresis and staining with ethidium bromide a major

branching enzyme I and about 80% identity to starch
branching enzyme II from other plant species. The present
inventors therefore denote the enzyme encoded by the new
branching enzyme sequence potato starch branching enzyme
5 II.

Transformation of potato plants

The isolated full length cDNA of potato starch
branching enzyme II and other functionally active frag-
ments in the range of 50-3 074 bp are cloned in reverse
10 orientation behind promoters active in potato tubers. By
the term "functionally active" is meant fragments that
will affect the amylose/amylopectin ratio in potato
starch. The DNA and amino acid sequence of SBE II
according to the invention as well as one fragment of the
15 DNA and corresponding amino acid sequence are shown in SEQ
ID No. 1 and 2, respectively.

The promoters are selected from, for example, the
patatin promoter, the promoter from the potato granule-
bound starch synthase I gene or promoters isolated from
20 potato starch branching enzymes I and II genes.

The constructs are cloned by techniques known in the
art either in a binary Ti-plasmid vector suitable for
transformation of potato mediated by *Agrobacterium*
tumefaciens, or in a vector suitable for direct
25 transformation using ballistic techniques or
electroporation. It is realized that the sense (see below)
and antisense constructs must contain all necessary
regulatory elements.

Transgenic potato plants transcribe the inverse
30 starch branching enzyme II construct specifically in
tubers, leading to antisense inhibition of the enzyme. A
reduction and changed pattern of the branching of amylo-
pectin as well as a changed amylose/amylopectin ratio
thereby occur in tuber starch.

35 The antisense construct for potato starch branching
enzyme II is also used in combination with antisense

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GCA ATT GAC AAG TAT GAG GGT GGT TTG GAA GCT TTT TCT CGT GGT TAT Ala Ile Asp Lys Tyr Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr 160 165 170	854
GAA AAA ATG GGT TTC ACT CGT AGT GCT ACA GGT ATC ACT TAC CGT GAG Glu Lys Met Gly Phe Thr Arg Ser Ala Thr Gly Ile Thr Tyr Arg Glu 175 180 185 190	902
TGG GCT CCT GGT GCC CAG TCA GCT GCC CTC ATT GGA GAT TTC AAC AAT Trp Ala Pro Gly Ala Gln Ser Ala Ala Leu Ile Gly Asp Phe Asn Asn 195 200 205	950
TGG GAC GCA AAT GCT GAC ATT ATG ACT CGG AAT GAA TTT GGT GTC TGG Trp Asp Ala Asn Ala Asp Ile Met Thr Arg Asn Glu Phe Gly Val Trp 210 215 220	998
GAG ATT TTT CTG CCA AAT AAT GTG GAT GGT TCT CCT GCA ATT CCT CAT Glu Ile Phe Leu Pro Asn Asn Val Asp Gly Ser Pro Ala Ile Pro His 225 230 235	1046
GGG TCC AGA GTG AAG ATA CGT ATG GAC ACT CCA TCA GGT GTT AAG GAT Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp 240 245 250	1094
TCC ATT CCT GCT TGG ATC AAC TAC TCT TTA CAG CTT CCT GAT GAA ATT Ser Ile Pro Ala Trp Ile Asn Tyr Ser Leu Gln Leu Pro Asp Glu Ile 255 260 265 270	1142
CCA TAT AAT GGA ATA TAT TAT GAT CCA CCC GAA GAG GAG AGG TAT ATC Pro Tyr Asn Gly Ile Tyr Tyr Asp Pro Pro Glu Glu Glu Arg Tyr Ile 275 280 285	1190
TTC CAA CAC CCA CGG CCA AAG AAA CCA AAG TCG CTG AGA ATA TAT GAA Phe Gln His Pro Arg Pro Lys Lys Pro Lys Ser Leu Arg Ile Tyr Glu 290 295 300	1238
TCT CAT ATT GGA ATG AGT AGT CCG GAG CCT AAA ATT AAC TCA TAC GTG Ser His Ile Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Val 305 310 315	1286
AAT TTT AGA GAT GAA GTT CTT CCT CGC ATA AAA AAG CTT GGG TAC AAT Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn 320 325 330	1334
CGC GTG CAA ATT ATG GCT ATT CAA GAG CAT TCT TAT TAT GCT AGT TTT Ala Val Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe 335 340 345 350	1382
GGT TAT CAT GTC ACA AAT TTT TTN GCA CCA AGC AGC CGT TTT GGA ACN Gly Tyr His Val Thr Asn Phe Xaa Ala Pro Ser Ser Arg Phe Gly Thr 355 360 365	1430
CCC GAC GAC CTT AAG TCT TTG ATT GAT AAA GCT CAT GAG CTA GGA ATT Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala His Glu Leu Gly Ile 370 375 380	1478
GTT GTT CTC ATG GAC ATT GTT CAC AGC CAT GCA TCA AAT AAT ACT TTA Val Val Leu Met Asp Ile Val His Ser His Ala Ser Asn Asn Thr Leu 385 390 395	1526
GAT GGA CTG AAC ATG TTT GAC GGC ACA GAT AGT TGT TAC TTT CAC TCT Asp Gly Leu Asn Met Phe Asp Gly Thr Asp Ser Cys Tyr Phe His Ser 400 405 410	1574

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TAT GAT TGA TGC AGA CGG AGA TTT GAC CTG GGA GAT GCA GAA TAT TTA Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu 675 680 685	2390
AGA TAC CGT GGG TTG CAA GAA TTT GAC CGG GCT ATG CAG TAT CTT GAA Arg Tyr Arg Gly Leu Gln Glu Phe Asp Arg Ala Met Gln Tyr Leu Glu 690 695 700	2438
GAT AAA TAT GAG TTT ATG ACT TCA GAA CAC CAG TTC ATA TCA CGA AAG Asp Lys Tyr Glu Phe Met Thr Ser Glu His Gln Phe Ile Ser Arg Lys 705 710 715	2486
GAT GAA GGA GAT AGG ATG ATT GTA TTT GAA AAA GGA AAC CTA GTT TTT Asp Glu Gly Asp Arg Met Ile Val Phe Glu Lys Gly Asn Leu Val Phe 720 725 730	2534
GTC TTT AAT TTT CAC TGG ACA AAA AGC TAT TCA GAC TAT CGC ATA GGC Val Phe Asn Phe His Trp Thr Lys Ser Tyr Ser Asp Tyr Arg Ile Gly 735 740 745 750	2582
TGC CTG AAG CCT GGA AAA TAC AAG GTT GCC TTG GAC TCA GAT GAT CCA Cys Leu Lys Pro Gly Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Pro 755 760 765	2630
CTT TTT GGT GGC TTC GGG AGA ATT GAT CAT AAT GCC GAA TAT TTC ACC Leu Phe Gly Gly Phe Gly Arg Ile Asp His Asn Ala Glu Tyr Phe Thr 770 775 780	2678
TTT GAA GGA TGG TAT GAT GAT CGT CCT CGT TCA ATT ATG GTG TAT GCA Phe Glu Gly Trp Tyr Asp Asp Arg Pro Arg Ser Ile Met Val Tyr Ala 785 790 795	2721
CCT AGT AGA ACA GCA GTG GTC TAT GCA CTA GTA GAC AAA GAA GAA GAA Pro Ser Arg Thr Ala Val Val Tyr Ala Leu Val Asp Lys Glu Glu Glu 800 805 810	2774
GAA GAA GAA GAA GTA GCA GTA GTA GAA GAA GTA GTA GTA GAA GAA GAA Glu Glu Glu Glu Val Ala Val Val Glu Glu Val Val Val Glu Glu Glu 815 820 825 830	2822
TGA ACGAA CTTGTGATCG CGTTGAAAGA TTTGAAGGCT ACATAGAGCT TCTTGACGTA ***	2880
TCTGGCAATA TTGCATCACT CTTGGCGGAA TTTTCATGTGA CAAAAGGTTT GCAATTCTTT CCACTATTAG TAGTGCAACG ATATACGCAG AGATGAAGTG CTGCACAAAC ATATGTAAAA TCGATGAATT TATGTCGAAT GCTGGGACGG GCTTCAGCAG GTTTTGCTTA GTGAGTTCTG TAAATTGTCA TCTC	2940 3000 3060 3074

TTC AAA TTT GAT GGA TTT AGA TTT GAT GGT GTG ACA TCA ATG ATG TAT	721
Phe Lys Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met Tyr	
225 230 235 240	
ACT CAC CAC GGA TTA TCG GTG GGA TTC ACT GGG AAC TAC GAG GAA TAC	769
Thr His His Gly Leu Ser Val Gly Phe Thr Gly Asn Tyr Glu Glu Tyr	
245 250 255	
TTT GGA CTC GCA ACT GAT GTG GAT GCT GTT GTG TAT CTG ATG CTG GTC	812
Phe Gly Leu Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu Val	
260 265 270	
AAC GAT CTT ATT CAT GGG CTT TTC CCA GAT GCA ATT ACC ATT GGT GAA	865
Asn Asp Leu Ile His Gly Leu Phe Pro Asp Ala Ile Thr Ile Gly Glu	
275 280 285	
GAT GTT AGC GGA ATG CCG ACA TTT TNT ATT CCC GTT CAA GAT GGG GGT	913
Asp Val Ser Gly Met Pro Thr Phe Xaa Ile Pro Val Gln Asp Gly Gly	
290 295 300	
GTT GGC TTT GAC TAT CGG CTG CAT ATG GCA ATT GCT GAT AAA TGG ATT	961
Val Gly Phe Asp Tyr Arg Leu His Met Ala Ile Ala Asp Lys Trp Ile	
305 310 315 320	
GAG TTG CTC AAG AAA CGG GAT GAG GAT TGG AGA GTG GGT GAT ATT GTT	1019
Glu Leu Leu Lys Lys Arg Asp Glu Asp Trp Arg Val Gly Asp Ile Val	
325 330 335	
CAT ACA CTG ACA AAT AGA AGA TGG TCG GAA AAG TGT GTT TCA TAC GCT	1057
His Thr Leu Thr Asn Arg Arg Trp Ser Glu Lys Cys Val Ser Tyr Ala	
340 345 350	
GAA AGT CAT GAT CAA GCT CTA GTC GGT GAT AAA ACT ATA GCA TTC TGG	1105
Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe Trp	
355 360 365	
CTG ATG GAC AAG GAT ATG TAT GAT TTT ATG GCT CTG GAT AGA CCN TCA	1153
Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro Ser	
370 375 380	
ACA TCA TTA ATA GAT CGT GGG ATA GCA TTG CAC AAG ATG ATT AGG CTT	1201
Thr Ser Leu Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg Leu	
385 390 395 400	
GTA ACT ATG GGA TTA GGA GGA GAA GGG TAC CTA AAT TTC ATG GGA AAT	1249
Val Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly Asn	
405 410 415	
GAA TTC GGC CAC CCT GAG TGG ATT GAT TTC CCT AGG GCT GAA CAA CAC	1297
Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg Ala Glu Gln His	
420 425 430	
CTC TCT GAT GGC TCA GTA ATT CCC GGA AAC CAA TTC AGT TAT GAT AAA	1345
Leu Ser Asp Gly Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp Lys	
435 440 445	
TGC AGA CGG AGA TTT GAC CTG GGA GAT GCA GAA TAT TTA AGA TAC CGT	1393
Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu Arg Tyr Arg	
450 455 460	

starch, characterized in that it comprises the following steps:

- a) transfer and incorporation of a vector according to claim 8 into the genome of a potato cell, and
- 5 b) regeneration of intact, whole plants from the transformed cells.

11. A process according to claim 10, wherein the vector also comprises an antisense construct of starch branching enzyme I (SBE I).

- 10 12. A process according to claims 10 or 11, wherein the vector also comprises an antisense construct of potato granule bound starch synthase II.

- 13. A process according to one or more of claims 10-12, wherein the vector also comprises an antisense
- 15 construct of potato soluble starch synthases II and III.

14. A process according to one or more of claims 10-13, wherein the vector also comprises an antisense construct of potato starch disproportionating enzyme (D-enzyme).

- 20 15. A process according to one or more of claims 10-14, wherein the vector also comprises an antisense construct of potato starch debranching enzyme.

16. A transgenic potato obtainable by the process according to any one of claims 9-15.

- 25 17. Use of transgenic potatoes according to claim 16 for the production of starch.

FIG. 2

Peptide 1. EFGVWEIFLPN

Peptide 2. HGLQEFDRA

Peptide 3. ENDGIAAKADE

Peptide 4. YEIDPEI/LTN

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SE 96/01558

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9504826	16/02/95	AU-A- 7535294	28/02/95
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		WO-A- 9211375	09/07/92

Form PCT/ISA/210 (patent family annex) (July 1992)



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 9/10, 15/82, A01H 5/06	A1	(11) International Publication Number: WO 97/20040 (43) International Publication Date: 5 June 1997 (05.06.97)
(21) International Application Number: PCT/SE96/01558 (22) International Filing Date: 28 November 1996 (28.11.96) (30) Priority Data: 9504272-7 29 November 1995 (29.11.95) SE 9601506-0 19 April 1996 (19.04.96) SE (71) Applicant (for all designated States except US): AMYLOGENE HB [SE/SE]; c/o Svalöf Weibull AB, S-268 81 Svalöv (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): EK, Bo [SE/SE]; Ny- hagen, S-740 30 Björklinge (SE). KHOSNOODI, Jamshid [SE/SE]; Bandstolsvägen 3, 2 tr., S-756 48 Uppsala (SE). LARSSON, Clas-Tomas [SE/SE]; Flogstavägen 55 B II, S- 752 73 Uppsala (SE). LARSSON, Håkan [SE/SE]; Hammar- bygatan 58, S-753 24 Uppsala (SE). RASK, Lars [SE/SE]; Säves väg 14, S-752 63 Uppsala (SE). (74) Agent: AWAPATENT AB; P.O. Box 5117, S-200 71 Malmö (SE).		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With a revised version of the international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the revised version of the international search report: 28 May 1998 (28.05.98)
(54) Title: STARCH BRANCHING ENZYME II OF POTATO (57) Abstract <p>The present invention relates to an amino acid sequence of second starch branching enzyme (SBE II) of potato and a fragment thereof as well as to the corresponding isolated DNA sequences. Furthermore, the invention relates to vectors comprising such an isolated DNA sequence, to processes for production of transgenic potatoes, and to the use of said potatoes for the production of starch. The starch obtained will show a changed pattern of branching of amylopectin as well as a changed amylose/amylopectin ratio.</p>		

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 96/01558

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/10, C12N 15/82, A01H 5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, BIOSIS, EMBL/GENBANK/DDBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9634968 A2 (NATIONAL STARCH AND CHEMICAL INVESTMENT HOLDING CORPORATION), 7 November 1996 (07.11.96) --	1-17
X	WO 9504826 A1 (INSTITUT FÜR GENBIOLOGISCHE FORSCHUNG BERLIN GMBH), 16 February 1995 (16.02.95), see abstract and claim 23 --	1-17
X	WO 9214827 A1 (INSTITUT FÜR GENBIOLOGISCHE FORSCHUNG BERLIN GMBH), 3 Sept 1992 (03.09.92), see page 5, line 1-7 and examples --	1-17

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Date of the actual completion of the international search

24 March 1998

Date of mailing of the international search report

01-04-1998

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Yvonne Siösteen

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/03/98

International application No.

PCT/SE 96/01558

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